

**Ecological drivers of *Mycobacterium avium* subsp. *paratuberculosis* detection in mongoose  
(*Herpestes ichneumon*) using IS900 as proxy**

Mónica V. Cunha<sup>1,2,3</sup>, Luís Miguel Rosalino<sup>2</sup>, Célia Leão<sup>1</sup>, Victor Bandeira<sup>4</sup>, Carlos Fonseca<sup>4</sup>, Ana Botelho<sup>1</sup>, Ana C. Reis<sup>1,2,3</sup>

<sup>1</sup>INIAV, IP- National Institute for Agrarian and Veterinary Research, Av. da República, Quinta do Marquês, 2780 -157 Oeiras, Portugal.

<sup>2</sup>Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa.

<sup>3</sup>Biosystems & Integrative Sciences Institute (BioISI), Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa.

<sup>4</sup>CESAM & Departamento de Biologia, Universidade de Aveiro, Portugal

**Supplementary Table 1.** Family, species, number and type of animal specimens processed for IS900 detection.

Family	Species	Total of animals	Total samples	Animals with matrices analyzed
<b>Canidae (n=40)</b>	Red fox ( <i>Vulpes vulpes</i> )	40	n= 26 F; n=29 S	16
<b>Mustelidae (n=8)</b>	Stone marten ( <i>Martes foina</i> )	4	n=3 F; n=3 S	2
	European badger ( <i>Meles meles</i> )	4	n=2 F; n=4 S	2
<b>Viverridae (n=5)</b>	Common genet ( <i>Genetta genetta</i> )	5	n=2 F; n=4 S	1
<b>Herpestidae (n=149)</b>	Egyptian mongoose ( <i>Herpestes ichneumon</i> )	149	n=78 F; n=109 S	37
<b>Total</b>		202	n=111 F; n=149 S	58

Legend: F - feces, S - spleen.

**Supplementary Table 2.** Mathematical formula for the calculation of diversity indices and non-parametric estimators.

	<b>Formula</b>
Shannon-Wiener (Help <i>et al.</i> , 1998)	$= -$ ,
Simpson (Help <i>et al.</i> , 1998)	$D = 1 -$
Berger-Parker (Help <i>et al.</i> , 1998)	$N\infty =$
Chao 1 (Gotelli and Cowell, 2011)	$S_{tot} = S_{obs} + (a^2 / 2b)$
Chao 2 (Gotelli and Cowell, 2011)	$S_{tot} = S_{obs} + (a^2 / 2b)$

Legend:  $p_i$  = proportional abundance of species  $i$  in the sample;  $p_1$  - proportional abundance of the most common species in the sample  $S_{obs}$  – total of observed species in our sample; Chao 1: a-singleton and b-doubleton, singleton and doubleton refers to abundance data, being the singletons the communities with one individual and doubleton the communities with two individuals; Chao 2: a-unique and b-duplicate, unique and duplicate, refers to incidence data, being unique the communities with one species and duplicates the communities with two species.

**Supplementary Table 3.** Diversity indices values by sampled district and NUTS (Statistical terrestrial units).

	Shannon-Wiener	Simpson	Berger-Parker
<b>Districts</b>			
Évora	0,691	0,498	1,875
Beja	0,153	0,058	1,030
Lisboa	0,349	0,198	1,125
Vila Real	1,168	0,640	2
Portalegre	0,655	0,463	1,571
Santarém	0,947	0,570	1,778
Coimbra	0,537	0,277	1,188
Castelo Branco	0,730	0,418	1,364
Guarda	0,562	0,375	1,333
Viseu	0,600	0,314	1,222
Aveiro	1,099	0,667	3
<b>NUTS</b>			
North	1,168	0,640	2,000
Center	0,899	0,457	1,408
Alentejo	0,540	0,326	1,253
Lisboa	0,349	0,198	1,125

Legend: North - Viana do Castelo, Braga, Porto, Vila Real and Bragança; Center – Aveiro, Viseu, Guarda, Castelo Branco, Leiria, Santarém and Coimbra; Alentejo – Portalegre, Évora, Beja and Setúbal.

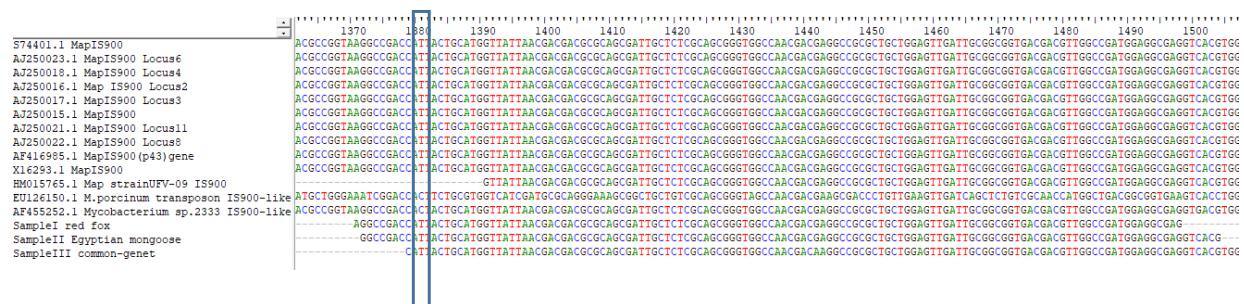
**Supplementary Table 4.** Demographic information of sampled and IS900-positive animals.

Geographic location	No of tested animals	No of positive animals	Cause of death	Age class	Year
Évora	<i>n</i> =13 HI <i>n</i> =16 VV	<i>n</i> =1 VV	<i>n</i> =10 H; 3 RK <i>n</i> =15 H; 1 RK	2 juveniles; 11 adults 3 cubs; 1 juvenile; 2 sub-adults; 7 adults; 3 NA	2010 ( <i>n</i> =10); 2011 <i>n</i> =3 2011 ( <i>n</i> =4); NA ( <i>n</i> =12)
Beja	<i>n</i> =66 HI <i>n</i> =1 VV <i>n</i> =1 MF	<i>n</i> =5 HI	<i>n</i> =66 H <i>n</i> =1 H <i>n</i> =1 RK	6 juveniles; 60 adults 1 adult 1 adult	2010 ( <i>n</i> =8); 2011 ( <i>n</i> =42); 2013 ( <i>n</i> =16) 2010 ( <i>n</i> =1) 2010 ( <i>n</i> =1)
Lisboa	<i>n</i> =8 HI <i>n</i> =1 VV	<i>n</i> =1 HI	<i>n</i> =8 H <i>n</i> =1 H	1 juvenile; 7 adults 1 adult	2009 ( <i>n</i> =1); 2010 ( <i>n</i> =7) NA ( <i>n</i> =1)
Vila Real	<i>n</i> =5 VV <i>n</i> =1 MM <i>n</i> =1 MF <i>n</i> =3 GG	<i>n</i> =1 VV	<i>n</i> =1H; 4 RK <i>n</i> =1 RK <i>n</i> =1 RK <i>n</i> =3 RK	1 juvenile; 4 adults 1 adult 1 adult 3 NA	2010 ( <i>n</i> =1); 2011 ( <i>n</i> =1); NA ( <i>n</i> =3) 2011 ( <i>n</i> =1) 2011 ( <i>n</i> =1) NA ( <i>n</i> =3)
Portalegre	<i>n</i> =7 HI <i>n</i> =4 VV	<i>n</i> =1 VV	<i>n</i> =7 H <i>n</i> =4 H	1 juvenile; 6 adults 4 adults	2008 ( <i>n</i> =1); 2010 ( <i>n</i> =3); 2011 ( <i>n</i> =3) 2010 ( <i>n</i> =4)
Santarém	<i>n</i> =9 HI <i>n</i> =5 VV <i>n</i> =2 MM		<i>n</i> =9H <i>n</i> =5 H <i>n</i> = 2 RK	1 juvenile; 8 adults 5 adults 1 cub; 1 adult	2010 ( <i>n</i> =2); 2011 ( <i>n</i> =7) 2011 ( <i>n</i> =4); NA ( <i>n</i> =1) 2011 ( <i>n</i> =2)
Coimbra	<i>n</i> =17 HI <i>n</i> =2 VV <i>n</i> =1 MF		<i>n</i> =17 H	3 juveniles; 11 adults; 3 NA 2 adults 1 adult	2008 ( <i>n</i> =2); 2010 ( <i>n</i> =2); 2011 ( <i>n</i> =7); 2013 ( <i>n</i> =3); 2014 ( <i>n</i> =3) 2006 ( <i>n</i> =1); 2010 ( <i>n</i> =1) NA ( <i>n</i> =1)
Castelo Branco	<i>n</i> =11 HI <i>n</i> =3 VV <i>n</i> =1 MM		<i>n</i> =10 H; 1 RK <i>n</i> =3 RK <i>n</i> =1 RK	1 juvenile; 10 adults 3 adults 1 adult	2009 ( <i>n</i> =3); 2010 ( <i>n</i> =6); 2011 ( <i>n</i> =1); 2012 ( <i>n</i> =1) 2010 ( <i>n</i> =1); 2011 ( <i>n</i> =2) NA (( <i>n</i> =1))
Guarda	<i>n</i> =3 HI <i>n</i> =1 VV		<i>n</i> =2 H; 1 RK <i>n</i> =1 RK	1juvenile; 2 adults 1 adult	2009 ( <i>n</i> =2); 2011 ( <i>n</i> =1) 2010 ( <i>n</i> =1)
Leiria	<i>n</i> =1 HI		<i>n</i> =1 H	1 adult	2011 ( <i>n</i> =1)
Viseu	<i>n</i> =9 HI <i>n</i> =1 VV <i>n</i> =1 GG	<i>n</i> =1 HI <i>n</i> =1 GG	<i>n</i> =9 H <i>n</i> =1 H <i>n</i> =1 RK	9 adults 1 adult 1 juvenile	2010 ( <i>n</i> =2); 2011 ( <i>n</i> =5); 2012 ( <i>n</i> =1); 2013 ( <i>n</i> =1) 2010 ( <i>n</i> =1) 2009 ( <i>n</i> =1)
Faro	<i>n</i> =5 HI	<i>n</i> =2 HI	<i>n</i> =4 H; 1 RK	5 adults	2011 ( <i>n</i> =5)
Aveiro	<i>n</i> =1 VV <i>n</i> =1 MF <i>n</i> =1 GG	<i>n</i> =1 VV <i>n</i> =1 MF	<i>n</i> =1 RK <i>n</i> =1 RK <i>n</i> =1 RK	1 adult 1 adult 1 adult	2011 ( <i>n</i> =1) 2005 ( <i>n</i> =1) NA
Total	<i>n</i> =149 HI <i>n</i> =40 VV <i>n</i> =4 MF <i>n</i> =4 MM <i>n</i> =5 GG	<i>n</i> =9 HI <i>n</i> =4 VV <i>n</i> =1 MF	<i>n</i> =173 H <i>n</i> =29 RK	<i>n</i> =4 cubs <i>n</i> =19 juveniles <i>n</i> =2 sub-adult <i>n</i> =168 adults <i>n</i> =9 NA	2005 ( <i>n</i> =1) 2006 ( <i>n</i> =1) 2008 ( <i>n</i> =3) 2009 ( <i>n</i> =7) 2010 ( <i>n</i> =43) 2012 ( <i>n</i> =2) 2011 ( <i>n</i> =99) 2013 ( <i>n</i> =20) 2014 ( <i>n</i> =3) NA ( <i>n</i> =23)

Legend- cause of death: H-hunted, RK-road-killed; NA - not available; VV: *Vulpes vulpes*; MF: *Martes foina*; MM: *Meles meles*; GG: *Genetta genetta*; HI: *Herpestes ichneumon*

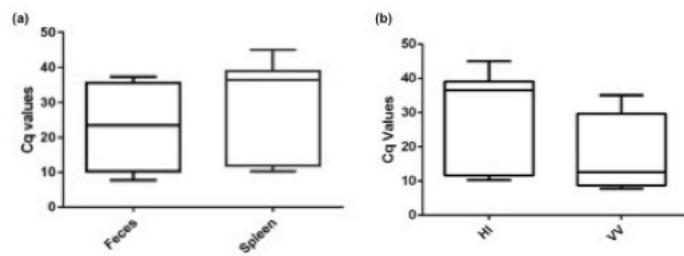


**Supplementary Figure 1.** The geographic distribution of the 202 animals under analysis across the districts of mainland Portugal is evidenced in grey scale.



This mutation can distinguish both IS900-like sequences from all of the IS900 sequences retrieved from NCBI, and from sequences obtained from sequencing of our samples by the amplification of a 224 bp fragment of IS900 with external primers.

**Supplementary Figure 2.** Nucleotide sequence alignment of three 224 bp IS900 amplicons from red fox, mongoose and genet (bottom), generated from the first PCR round, with 11 IS900 sequences from 11 MAP strains genomes (Accession numbers AJ250023.1, AJ250018.1, AJ250016.1, AJ250017.1, AJ250015.1, AJ250021.1, AJ250022.1, S74401.1, AF416985.1, X16293.1 and HM015765.1) and two IS900-like sequences from *Mycobacterium* sp. 2333, accession number AF455252.1 and *Mycobacterium porcinum*, accession number EU126150.1. The SNP (T>C) that distinguishes IS900-like from MAP IS900 is highlighted.



**Supplementary Figure 3.** Distribution of the Cq values obtained by semi-nested real-time IS900 PCR for feces and spleen samples (a); and for Egyptian mongoose and red foxes samples (b). HI - *Herpestes ichneumon* and VV - *Vulpes vulpes*.